

Applicant : Steven Reppert et al.

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Examiner : M. Pak

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Title : HIGH-AFFINITY MELATONIN RECEPTOR AND USES THEREOF

PROPOSED AMENDED CLAIMS FOR DISCUSSION PURPOSES ONLY

1-52. (cancelled)

53. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein comprising an amino acid ~~sequence substantially identical~~ at least 80% identical in amino acid sequence to SEQ ID NO:12, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;

b) measuring intracellular cAMP concentration in said cell; and

c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

54-77. (cancelled)

78. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein comprising an amino acid ~~sequence substantially identical~~ at least 80% identical to that of SEQ ID NO:6, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;

b) measuring intracellular cAMP concentration in said cell; and
c) where said contacting causes a decrease in intracellular cAMP concentration,
identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

79. (cancelled)

80. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein, wherein the expression vector comprises a sequence that hybridizes to a probe having the sequence of the complement of SEQ ID NO:5 under the following conditions: hybridization in 50% formamide, 1 M sodium chloride, 1% SDS, 10% dextran sulfate, 100 µg/ml denatured salmon sperm at 42 °C, and filters washed in 2x SSC, 1% SDS at 65 °C for 1 hour;

b) measuring intracellular cAMP concentration in said cell; and
c) where said contacting causes a decrease in intracellular cAMP concentration,
identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

81. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein, wherein the expression vector comprises a sequence that hybridizes to a probe having the sequence of the complement of SEQ ID NO:5 under the following conditions: hybridization in 25% formamide, 1 M sodium chloride, 1% SDS, 10% dextran sulfate, 100 µg/ml denatured salmon sperm at 42 °C, and filters washed in 2x SSC, 1% SDS at 55 °C for 1 hour;

b) measuring intracellular cAMP concentration in said cell; and
c) where said contacting causes a decrease in intracellular cAMP concentration,
identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

82. (previously presented) The method of claim 81, wherein the expression vector comprises the sequence of SEQ ID NO:5.

83. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

- a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein, wherein the expression vector comprises a sequence that hybridizes to a probe having the sequence of the complement of SEQ ID NO:11 under the following conditions: hybridization in 50% formamide, 1 M sodium chloride, 1% SDS, 10% dextran sulfate, 100 µg/ml denatured salmon sperm at 42 °C, and filters washed in 2x SSC, 1% SDS at 65 °C for 1 hour;
- b) measuring intracellular cAMP concentration in said cell; and
- c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

84. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

- a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein, wherein the expression vector comprises a sequence that hybridizes to a probe having the sequence of the complement of SEQ ID NO:11 under the following conditions: hybridization in 25% formamide, 1 M sodium chloride, 1% SDS, 10% dextran sulfate, 100 µg/ml denatured salmon sperm at 42 °C, and filters washed in 2x SSC, 1% SDS at 55 °C for 1 hour;
- b) measuring intracellular cAMP concentration in said cell; and
- c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand. --

85. (previously presented) The method of claim 84, wherein the expression vector comprises the sequence of SEQ ID NO:11.

86. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

- a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein that consists of the amino acid sequence of SEQ ID NO:12, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;
- b) measuring intracellular cAMP concentration in said cell; and
- c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

87. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

- a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein comprising the amino acid sequence of SEQ ID NO:6, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;
- b) measuring intracellular cAMP concentration in said cell; and
- c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

88. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

- a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein that comprises the amino acid sequence of SEQ ID NO:12, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;
- b) measuring intracellular cAMP concentration in said cell; and
- c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

89. (currently amended) A method of testing a candidate compound for the ability to act as an agonist of a ~~high-affinity~~ melatonin receptor ligand, said method comprising:

a) contacting said candidate compound with a cell comprising an expression vector encoding a ~~high-affinity~~ melatonin receptor protein consisting of the amino acid sequence of SEQ ID NO:6, or a melatonin binding fragment thereof, wherein the cell expresses on its surface said ~~high-affinity~~ melatonin receptor protein or melatonin binding fragment thereof;

b) measuring intracellular cAMP concentration in said cell; and

c) where said contacting causes a decrease in intracellular cAMP concentration, identifying said candidate compound as an agonist of a ~~high-affinity~~ melatonin receptor ligand.

90. (new) The method of claim 53, wherein the melatonin receptor protein differs from SEQ ID NO:12, or a melatonin binding fragment thereof, only by conservative substitutions.

91. (new) The method of claim 78, wherein the melatonin receptor protein differs from SEQ ID NO:6, or a melatonin binding fragment thereof, only by conservative substitutions.